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Rumen Microbial Protein Synthesis and Milk Performance in Lactating Dairy Cows Fed the Fortified Corn Stover Diet in Comparison with Alfalfa Diet

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Abstract: The objective of this study was to compare the rumen microbial protein synthesis and milk performance in lactating dairy cows fed the fortified corn stover diet with alfalfa diet. Twelve multiparous Holstein lactating cows were used in a 3×3 Latin square design involving three diets with different forage ingredients: Alfalfa (AH), Corn Stover Supplemented with starch (CSS) and CSS supplemented with rumen-protected methionine and lysine (CSSAA). The basal diets were isonitrogenous and isocaloric with a ratio of forage to concentrate of 45:55 (Dry Matter (DM) basis). The daily DM intake ($p = 0.68$) and milk yield ($p = 0.20$) did not differ among the treatments. Compared with CSS, supplementation of Rumen Protected Amino Acid (RPAA) increased the 4% fat-corrected milk ($p = 0.06$) and milk efficiency ($p = 0.08$). Milk protein yield was higher ($p = 0.07$) for cows fed AH than those fed CSS with CSSAA at an intermediate position. Ruminal ammonia nitrogen concentrations ($p<0.01$) for AH diet was lower than for CSS and CSSAA. Total volatile fatty acids concentration ($p = 0.65$), microbial protein yield ($p = 0.71$) and metabolizable protein ($p = 0.61$) did not differ among the treatments. Lower N conversion ($p<0.01$) was detected for CSS than AH and CSSAA. It is indicated that supplementation of starch and amino acid to a corn stover diet achieve a similar lactation performance as the cows fed alfalfa diet.

Key words: Corn stover, starch, rumen-protected amino acid, lactation performance, dairy cow

INTRODUCTION

In China, >100×10^9 kg of Corn Stover (CS) is generated annually (Pang *et al*., 2008); however, using CS as a forage source for ruminants has been limited due to its deficiency in key nutrients. In the earlier study involving the comparison of CS and alfalfa diets in dairy cows (Zhu *et al*., 2013), ruminal energy fermentation was insufficient for Microbial Protein (MCP) synthesis in the rumen with CS which resulted in a decreased supply of Metabolizable Protein (MP) and consequently, a lower yield of milk protein. The easily fermentable carbohydrate in CS diet was lower than alfalfa diet depending on the analysis by Cornell Net Carbohydrate and Protein System (CNCPS) Model. The supplementation of readily fermentable carbohydrates may be beneficial for the effective capture of RDP and increase the MP supply for milk protein synthesis. Starch as a major of energy source in most dairy cows feedstuff, accounting for 50-100% of Non-structure Carbohydrate (NRC, 2001) and an increased milk yield and Nitrogen (N)-utilization efficiency may be expected with increasing non-structure carbohydrate intake, particularly corn starch, in lactating cows (Rius *et al*., 2010).

The >90% of milk protein is synthesized from free Amino Acid (AA) in the blood as the precursors and the AA available for absorption are influenced by the AA composition of MCP (Piepenbrink *et al*., 1998) and RUP (Romagnolo *et al*., 1994). The profile of AA supplied by MCP is relatively constant (Allen, 2000) such that the supplementation of Rumen-Protected (RP) AA can help to optimize the AA pool for protein synthesis. In the earlier study (Zhu *et al*., 2013), the data for urinary N indicated that CS might have a poor AA balance compared with alfalfa. Because the lysine and methionine are considered to be the most limiting AA for milk protein synthesis in dairy cows (NRC, 2001), improving the balance of these AA is an effective approach to enhance milk protein production and improve milk protein synthesis efficiency (Cho *et al*., 2007).

Therefore, the objective of this study was compare the rumen microbial protein synthesis and milk performance in lactating dairy cows fed the corn stover diet fortified by starch and AA with alfalfa diet.

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MATERIALS AND METHODS

Animals, diets and experimental design: All the procedures involving the use of animals were approved by the Animal Care Committee, Zhejiang University, Hangzhou, China. Twelve multiparous (3.5±1.0 parity) Chinese Holstein cows (661±46.3 kg Body Weight (BW); 133±13.5 days in milk; 31±3.3 kg day^{-1} milk yield) were used. The cows were divided into three groups of four each according to the similarity of their day in milk and milk yield values and then were randomly allocated in a multiple 3×3 Latin square.

Three isonitrogenous and isocaloric basal diets were formulated to meet the NE_L requirements of cows for a milk production value of 32 kg day^{-1} (MOA and China, 2004). The ingredients and chemical composition of the diets are shown in Table 1 and 2, respectively. All the diets had a ratio of forage to concentrate of 45:55 (DM basis) with a similar component of concentrate. The diets contained the following forage ingredients (% DM basis): alfalfa, 19 and Chinese wild rye grass, 6 (AH); corn starch, 8, CS, 22 and alfalfa, 6 (CSS) and CSS supplemented with 19.7 g RPMethionine and 40.1 g RPLysine (CSSAA). All the diets contained 17% corn silage. Both RPMethionine and RPLysine were commercial products (Hangzhou King Techina Co. Ltd., China). The supplemental RPAA was expected to provide 10.6 and 11.2 g/cow/day of intestinal digestible methionine and lysine, respectively as based on the manufacturer's specifications. The CPM Dairy (Cornell-Penn-Miner version of CNCPS) prediction for the proportion of methionine and lysine in MP was 2.4 and 7.2% for the CSSAA diet.

Table 1: Ingredients of the experimental diets

	Treatments[1]		
Item (On a DM basis %)	AH	CSS	CSSAA
Corn silage	17.00	17.00	17.00
Corn stover	0.00	22.00	22.00
Chinese wild ryegrass hay	9.00	0.00	0.00
Alfalfa hay	19.00	6.00	6.00
Ground corn grain	15.02	12.02	12.02
Corn starch	0.00	8.00	8.00
Soybean meal (45.4% CP)	5.03	9.47	9.47
Cottonseed meal	1.74	3.72	3.72
Wheat	2.12	2.12	2.12
Barley	7.22	7.11	7.11
Expanded soybean	0.91	2.90	2.90
DDGS	2.16	0.00	0.00
Soybean hulls	2.05	0.00	0.00
Double-low rapeseed meal	3.14	2.00	2.00
Cotton seed	4.07	1.74	1.74
Beet pulp	7.21	0.00	0.00
Premix[2]	0.46	0.46	0.46
Salt	0.46	0.46	0.46
Dicalcium phosphate	0.48	0.50	0.50
Limestone	0.65	1.20	1.20
Sodium bicarbonate	0.60	0.60	0.60
Ca salts of long-chain fatty acids	1.59	1.70	1.70
Urea	0.08	1.00	1.00
Methionine (g day^{-1})[3]	0.00	0.00	19.73
Lysine (g day^{-1})[4]	0.00	0.00	40.14

[1]AH = Diet containing alfalfa hay as the main forage; CSS = Diet containing corn stover as the main forage with starch supplementation; CSSAA = CSS supplemented with rumen-protected Methionine and Lysine. [2]Formulated to provide (per kg of DM) 500,000-700,000 IU of vitamin A, 140,000-170,000 IU of vitamin D3, 2,000-4,000 IU of vitamin E, 7,000-9,000 mg of Zn, 40-80 mg of Se, 180 mg of I, 1,400-2,500 mg of Fe, 15-30 mg of Co, 1,4000-2,500 mg of Mn and 1,400-2,500 mg of Cu. [3]Contained 60% Methionine with a 93.8% rumen-protected ratio and was provided by Hangzhou King Techina Co. Ltd., China. [4]Contained 60% Lysine with a 93.0% rumen-protected ratio and was provided by Hangzhou King Techina Co., Ltd. China

Each experimental period consisted of 21 days with the first 16 days used as an adaptation period. The diets were fed as Total Mixed Ratio (TMR) with at least 5-10% orts. The daily feed was offered 3 times per day at 600, 1230 and 1930 h. The cows were individually housed in a tie-stall barn and milked 3 times daily at 630, 1430 and 2030 h. All the cows had free access to drinking water throughout the experiment.

Sampling, measurement and analyses: The DM content of the forages and concentrates was determined weekly to adjust their allocation to maintain a consistent ratio of forage to concentrate on a DM basis. The DM Intake (DMI) determination and sample preparation and analysis were performed by methods described earlier (Zhu *et al*., 2013). All the samples were analyzed for DM, Organic Matter (OM), Crud Protein (CP) and Ether Extract (EE) (AOAC, 1990) for Neutral Detergent Fiber (NDF) and Acid Detergent Fiber ADF (Van Soest *et al*., 1991) for sucrose (Miller-Webster and Hoover, 1998) and for starch using a Colorimetric Method (Bertrand *et al*., 2003).

Table 2: Chemical composition of the experimental diets[1]

	Treatment [2]		
Composition	AH	CSS	CSSAA
OM (DM %)	91.6±0.10	91.0±0.13	91.0±0.13
NDF (DM %)	36.1±0.07	36.8±0.05	36.8±0.05
NFC[3] (DM %)	36.7±0.07	36.2±0.06	36.2±0.06
Sucrose (DM %)	7.5±0.01	6.7±0.01	6.7±0.01
Starch (DM %)	21.5±0.04	26.9±0.05	26.9±0.05
CP (DM %)	15.4±0.02	15.4±0.10	15.5±0.10
Lysine (MP[4] %)	6.70	6.83	7.20
Methionine (MP[4] %)	1.98	1.97	2.40
Lysine:methionine	3.38:1	3.47:1	3.00:1
Ca (DM %)[5]	0.87	0.87	0.87
P (DM %)[5]	0.44	0.43	0.43
NEL (Mcal kg^{-1} of DM)[5]	1.58	1.58	1.58

[1]The chemical compositions of the main forage were as follows (of % DM): corn silage-OM = 91.2, CP = 7.3, NDF = 57.5 and ADF = 32.2; corn stover OM = 84.9, CP = 6.2, NDF = 68.9 and ADF = 37.1; Chinese wild ryegrass hay OM = 88.7, CP = 7.7, NDF = 70.1 and ADF = 32.1 and alfalfa hay-OM = 89.0, CP = 19.0 = NDF = 40.1 and ADF = 31.4.[2] AH = diet containing alfalfa hay as the main forage; CSS = Diet containing corn stover as the main forage with starch supplementation; CSSAA = CSS supplemented with rumen-protected Methionine and Lysine. [3]NFC = non-fiber carbohydrate = 100-NDF %-CP %-ether extract %-ash %. [4]Calculated by the CPM (Cornell-Penn-Miner version of CNCPS) dairy prediction software. [5]Calculated based on MOA and China (2004)

On day 17, 18 and 19 of each period, the milk yield was recorded and milk samples were collected; blood was sampled on day 20. Analysis of milk composition and the preparation of plasma were according to methods described by Zhu *et al.* (2013). Analysis of Milk Urea Nitrogen (MUN) and Plasma Urea Nitrogen (PUN) was according to Wang *et al.* (2010).

On day 21 of each period, rumen fluid samples (approximately 100 mL) were collected by the oral stomach tube (Anscitech Co., Ltd. Wuhan, China) from oral cavity at 2 h after feeding according to methods described by Shen *et al.* (2012). Collections were squeezed through 4 layers of cheesecloth and pH of the filtered ruminal fluid was measured immediately using a portable pH meter (Starter 300; Ohaus Instruments Co., Ltd. Shanghai, China). Two (5.0 mL) aliquots of each rumen filtrates were collected and stored at -20°C and subsequently analyzed for Volatile Fatty Acids (VFA) and ammonia nitrogen (NH_3-N). One subsample was acidified with 1.0 mL of 25% orthophosphoric acid for VFA determination. All rumen fluid subsamples were thawed and centrifuged at 4°C before analysis. The acidified subsamples were centrifuged at 20,000×g for 10 min and supernatants (1.0 mL) were measured for VFA by gas chromatography (GC-8A; Shimadzu Corp., Kyoto, Japan) according to the method described by Hu *et al.* (2005). Other subsamples were centrifuged at 4,000×g for 10 min and the supernatants (2.0 mL) were used to determine the NH_3-N as described by Hu *et al.* (2005). The BW for all the cows was measured before the morning feeding at the beginning and end of each period.

Microbial protein and MP: The MP supply was calculated as the sum of the Intestinally Absorbable Digestible Dietary Protein (IADP) and Intestinally Absorbable MCP (IAMCP). The Intestinal Digestibility of RUP (IDP) was determined according to the modified 3 step procedure of Gargallo *et al.* (2006). For the determination of RUP, the degradation of OM and CP of TMR were measured using the nylon bag technique. Briefly, three ruminally cannulated multiparous cows were used for the determination of the rumen OM and CP degradation of TMR. The data for the disappearance of OM and CP from the nylon bag were fitted to the model of Orskov and McDonald (1979) to estimate the *in situ* degradation constants and the Effective Rumen Degradability (ERD) was calculated.

The MCP synthesis in the rumen was estimated by the urinary Purine Derivative (PD) value as reported by Chen and Gomes (1992). The collection and pretreatment of the urine samples was as described by Zhu *et al.* (2013). The IAMCP value was estimated as MCP yield× 0.64 (NRC, 2001).

Statistical analysis: The statistical analyses were performed using SAS Software (SAS, 2000). The Statistical analyses on DMI, milk yield and composition, milk efficiency, MCP yield, MUN and urinary N, BW gain and PUN were conducted using MIXED procedure with covariance type AR (1). Data for DMI, milk yield and composition, milk efficiency, MCP yield, MUN and urinary N were averaged per cow and period. Data were analyzed as a multiple Latin square and the statistical model used was:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

Where:

Y_{ijk} = The dependent variable
μ = The overall mean
C_i = The effects of cow
P_j = The effects of period
T_k = The effects of treatment
e_{ijk} = The random error term

The effect of period and treatment were considered fixed and cow as a random effect. The least square means were calculated and separated using the PDIFF option and the differences between the treatments were detected using Tukey's adjustment.

The data for the constants of the OM and CP degradation in the rumen (a, b and c) ERD, RUP and intestinal digestion parameters were analyzed using a GLM procedure. All the results were reported as LSMEANS. A statistically significant difference and a trend were considered at $p<0.05$ and $0.05 \le p \le 0.10$, respectively.

RESULTS AND DISCUSSION

Feed intake, milk yield and composition: The daily DMI did not differ (p = 0.68) among the treatments with an average of 20.7 kg day^{-1} (Table 3) and the milk yield was not affected (p = 0.20) by the treatment with an average of 28.6 kg day^{-1}. The supplementation of RPAA to the CSS diet increased (p = 0.06) the 4% fat-corrected milk from 26.9-28.6 kg day^{-1}. The supplementation of the CSS diet with RPAA increased by 1.4 kg day^{-1} milk yield resulting in an increasing milk efficiency (milk yield/DMI = 1.34 vs. 1.43, p = 0.08) with no difference between AH and CSSAA or CSS.

The milk protein content did not differ (p = 0.29) among the treatments and ranged from 3.01-3.09% whereas the cows fed the CSS diet produced milk that was lower (p = 0.07) in protein yield in comparison with those

Table 3: The DM Intake (DMI) and milk production of dairy cows fed the corn stover diet fortified with starch and amino acids in comparison with alfalfa diet

	Treatments[1]				
Items	AH	CSS	CSSAA	SEM[2]	p-value
DMI (kg day^{-1})	21.000	20.700	20.500	0.410	0.68
Milk production (kg day^{-1})					
Milk yield	29.000	27.700	29.100	0.770	0.20
4% FCM[3]	28.100	26.900	28.600	0.660	0.06
Milk protein	0.895	0.833	0.891	0.023	0.07
Milk composition (%)					
Protein	3.090	3.010	3.060	0.034	0.29
Fat	3.810	3.810	3.900	0.050	0.27
Lactose	4.860	4.810	4.850	0.038	0.34
BW gain (g day^{-1})	152.000	136.000	142.000	7.100	0.27
Milk efficiency[4]	1.400	1.340	1.430	0.032	0.08

[a, b]Means within the same row with different superscripts differ at p<0.05. [1]AH = Diet containing alfalfa hay as the main forage; CSS = Diet containing corn stover as the main forage with starch supplementation; CSSAA = CSS supplemented with rumen-protected Methionine and Lysine. [2]Standard error of means. [3]4% FCM = Fat-Corrected Milk = milk production× (0.4+0.15×milk fat content). [4]Milk efficiency = milk yield/DMI

Table 4: Rumen fermentation characteristics of dairy cows fed the corn stover diet fortified with starch and amino acids in comparison with alfalfa diet

	Treatment[1]				
Items	AH	CSS	CSSAA	SEM[2]	p-value
pH	6.61	6.63	6.58	0.069	0.87
Ammonia nitrogen (mg dL^{-1})	11.20^{b}	12.70^{a}	13.00^{a}	0.370	<0.01
Total VFA (mM)	81.90	83.90	84.00	1.990	0.65
Acetate (mol/100 mol)	72.40	72.00	72.20	0.190	0.63
Propionate (mol/100 mol)	17.40^{b}	17.90^{a}	17.40^{b}	0.160	0.01
Butyrate (mol/100 mol)	10.40	10.10	10.40	0.140	0.21
Acetate:propionate	4.17^{a}	4.04^{b}	4.17^{a}	0.040	0.04

[a, b]Means within the same row with different superscripts differ at p<0.05; [1]AH = Diet containing alfalfa hay as the main forage; CSS = Diet containing corn stover as the main forage with starch supplementation; CSSAA = CSS supplemented with rumen-protected methionine and lysine. [2]Standard error of means

fed the AH and CSSAA diets (Table 3). The dietary treatment had no effect on the fat (p = 0.27) and lactose (p = 0.34) content of the milk. The change in BW was not affected by the treatment (p = 0.27).

Rumen fermentation, microbial protein and MP: No difference was detected among treatments for pH (p = 0.87), total VFA (p = 0.65) and the molar proportion of acetate (p = 0.63) and butyrate (p = 0.21) (Table 4). The NH_3-N concentration was lower (p<0.01) in cows fed AH than those fed CSS or CSSAA with no difference between CSS and CSSAA. The molar proportion of propionate in cows fed diet CSS was higher (p = 0.01) than those fed AH or CSSAA, resulting in lower (p = 0.04) acetate to propionate ratio for CSS than that for AH or CSSAA with no difference between AH and CSSAA.

The results for OM and CP degradation and RUP are shown in Table 5. For OM degradation, the CSS and CSSAA diets exhibited higher a (p<0.01) and lower b (p<0.01) values in comparison to AH; the c values showed no difference among the treatments (p = 0.93). The ERD value of OM did not differ (p = 0.16) among the treatments with an average of 55.6%. For CP degradation, compared with the AH diet, the CSS and CSSAA diets resulted in higher a (p<0.01) values, although the b (p<0.01) and c (p = 0.03) values were lower. The ERD values of CP for the CSS and CSSAA diets were lower (p = 0.04) than AH thus the RUP for CSS and CSSAA were higher (p = 0.04) than that for AH (Table 5).

Table 5: The OM and CP degradation constants based on the equation p = a+b[1-exp(-ct)], their effective degradability and rumen-undegradable protein of the experimental diets

	Treatments[1]				
Items	AH	CSS	CSSAA	SEM[2]	p-value
OM degradation					
a (%)	34.5^{b}	38.3^{a}	38.2^{a}	0.350	<0.01
b (%)	59.2^{a}	52.9^{b}	52.4^{b}	0.700	<0.01
c (% h^{-1})	4.16	4.11	4.06	0.165	0.93
ERD[3]	54.8	56.1	56.0	0.470	0.16
CP degradation					
a (%)	18.0^{b}	22.3^{a}	21.4^{a}	0.580	<0.01
b (%)	71.7^{a}	65.6^{b}	66.4^{b}	0.420	<0.01
c (% h^{-1})	7.54^{a}	6.28^{b}	6.40^{b}	0.278	0.03
ERD[3]	52.7^{a}	51.1^{b}	50.9^{b}	0.450	0.04
RUP (% CP)[4]	47.3^{b}	48.9^{a}	49.1^{a}	0.450	0.04

[a, b]Means within the same row with different superscripts differ at p<0.05; [1]AH = Diet containing alfalfa hay as the main forage; CSS = Diet containing corn stover as the main forage with starch supplementation; CSSAA = CSS supplemented with rumen-protected Methionine and Lysine. [2]Standard error of means. [3]ERD = Effective Rumen Degradability = a + bc/(c + kp) (Orskov and McDonald, 1979), assuming 8% h^{-1} as the passage rate (kp) (Madsen and Hvelplund, 1985). [4]RUP = 100-RDP

No difference was observed among the treatments for IDP (p = 0.85) and IADP (p = 0.95) (Table 6) and the MCP yield did not differ (p = 0.71) among the treatments. Therefore, no effects were detected among the treatments with regard to MP (p = 0.71).

Nitrogen utilization: Compared with AH and CSSAA, the CSS diet resulted in a lower (p<0.01) N conversion (milk N/N intake) with no difference between AH and CSSAA (Table 6). The concentration of urinary urea N with the CSS diet was higher (p = 0.03) than AH with CSSAA being intermediate. The urea N concentration in the milk and plasma of the cows fed the CSSAA diet was lower (p = 0.02) than that of the cows fed CSS with a medium value for AH.

In the present study, starch supplementation increased the a value for the CSS diet and the higher a AH diet (Table 5). A high relationship exists between VFA value compensated for the negative effects of a lower b value, resulting in a similar ruminal OM degradation as the

Table 6: Supplementation on the Metabolizable Protein (MP) supply, Nitrogen (N) utilization efficiency and urea N concentration of dairy cows fed the corn stover diet fortified with starch and amino acids in comparison with alfalfa diet

	Treatments[1]				
Items	AH	CSS	CSSAA	SEM[2]	p-value
IDP (% of RUP[3])	73.1	69.9	72.6	0.85	0.85
IADP (g day^{-1})[4]	1115	1089	1133	22.0	0.90
MCP (g day^{-1})[5]	1948	1887	1908	58.7	0.71
IAMCP (g day^{-1})[6]	1247	1208	1221	42.8	0.61
MP (g day^{-1})[7]	2362	2297	2354	67.7	0.61
N conversion[8]	0.279^{a}	0.262^{b}	0.282^{a}	0.0033	<0.01
Urea N concentration (mg dL^{-1})					
Blood	15.0^{ab}	16.2^{a}	14.6^{b}	0.40	0.03
Urine	425^{b}	485^{a}	448^{ab}	15.3	0.03
Milk	14.8^{ab}	15.5^{a}	14.5^{b}	0.26	0.02

$^{a, b}$Means within the same row with different superscripts differ at p<0.05; [1]AH = TMR containing alfalfa hay as the main forage; CSS = TMR containing corn stover as the main forage with starch supplementation; CSSAA = CSS supplemented with rumen-protected Methionine and Lysine. [2]Standard error of means. [3]IDP = Measured intestinal digestibility of RUP, according to a modified 3-step procedure (Gargallo *et al*., 2006). [4]IADP = Intestinally Absorbable Dietary Protein = RUP×CP intake×IDP. [5]MCP = Microbial Protein, estimated based on the purine derivatives according to Chen and Gomes (1992). [6]IAMCP = Intestinally Absorbable MCP = MCP×0.64 (NRC, 2001). [7]MP = IAMCP+IADP. [8]N conversion = Milk protein yield/CP intake

production and OM degradation in the rumen (Menke and Steingass, 1988). No difference in VFA concentration was detected among the treatments (Table 4). Ruminal VFA is served as the main energy source to the dairy cows (Russell and Hespell, 1981) and is proportional to their production. Similar milk yield (Table 3) observed among the treatments partly due to similar ruminal VFA concentration (Table 4).

Starch acts as a source of fermentable OM for MCP synthesis and may increase the amount of MCP reaching the small intestine in ruminants (Jarrige, 1989). The ruminal degradation of OM is the predominant factor contributing to MCP yield (Clark *et al*., 1992). Thus, no difference in MCP yield and MP supply was detected among treatments (Table 6). However, the MCP yield for CSS was 3.1% lower than that for AH which contributed the 2.8% lower MP supply to cows fed CSS. The rumen degradation rate of CP in cows fed CS was 6.5% h (Zhu *et al*., 2013), though corn starch degrades at a rate of approximately 4.0-6.4% h. Thus, a partial replacement of corn starch with starch from barely, oat or wheat (rapidly degraded carbohydrates, NRC (2001) may provide a more synchronous supply of energy and N in cows fed CS than those fed only with corn starch supplementation. The lower RDP and higher ruminal NH_3-N also indicated the unsynchronization of the rate of carbohydrate fermentation and protein degradation in diet CSS and CSSAA compared with AH.

Milk protein secretion in dairy cows is closely associated with the MP supply (NRC, 2001; Zhu *et al*., 2013). However, cows fed diet CSSAA had 2.5% higher MP supply (Table 6) demonstrated a 7.0% higher milk protein yield (Table 3) than those fed CSS. These observations indicated that RPAA supplementation improves the conversion efficiency of AA into protein (MP utilization efficiency). In terms of metabolism, the MP-utilization efficiency is influenced by the AA used for protein synthesis and AA catabolism generates urea (Lapierre *et al*., 2002). Thus, balancing diets deficient in lysine and methionine could increase the efficiency of MP utilization (NRC, 2001). Beckman and Weiss (2005) reported that high starch with adequate available MP can increase the milk protein yield, whereas high starch with low MP should not have the same effect. In the present study, when estimated by CPM Dairy, the CSSAA diet was adequate with regard to MP. The MP-allowable milk yield was 29.4 kg day^{-1} in comparison with the observed 29.1 kg day^{-1} milk yield. The predicted lysine and Methionine in MP were at an appropriate ratio (3:1) and had optimal concentrations and proportions (7.2-2.4%). Similar results were observed in earlier studies (Lee *et al*., 2012; Robinson, 2010). The milk protein content was not affected by RPAA supplementation whereas the milk protein yield was increased.

The excessive ruminal NH_3-N will be absorbed through the rumen wall in to blood and be converted into urea in the liver, the urea will be excreted in the rumen, urine and milk finally (Linn and Olsen, 1995). Compared with diet CSS, CSSAA had lower urea N in blood and milk (Table 6). This information together with the similar ruminal NH_3-N concentration between them (Table 4) indicated higher NH_3-N utilization efficiency in CSSAA. Wang *et al*. (2010) also reported that dairy cows fed diets containing balanced AA increased the efficiency of N utilization. Compared with diet CSS, the decreased concentration of plasma urea N in CSSAA indicated that RPAA supplementation could improve the AA balance in MP and resulted in less deamination of the absorbed AA. A low plasma urea N concentration with RPAA supplementation is beneficial for animal health, particularly for reproduction whereas a high plasma urea N concentration will decrease embryo viability (Rhoads *et al*., 2006).

CONCLUSION

Supplementation of starch to a corn stover diet provided more readily fermentable carbohydrates in the rumen and achieving similar MCP synthesis and MP supply with those by the AH diet. The further supplementation of RPAA improved the AA balance and increased the efficiency of N utilization in starch-supplemented corn stover diet.

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